

# **The role of the ERLIN complex in regulating the morphology of the endoplasmic reticulum**

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## Abstract

The endoplasmic reticulum (ER) is an interconnected, continuous membrane system reaching throughout the cell, forming connections with almost all organelles in the cell. The communication facilitated by the ER is essential for many cellular functions and ultimately for cell survival. To achieve that, the ER needs to sustain a dynamic morphology in order to be able to adjust its shape in response to internal and external stimuli. This includes the maintenance and the formation of specific domains like ER-sheets and ER-tubules to ensure cellular integrity.

ERLIN1 and ERLIN2 are two homologous proteins that localize to the ER membrane. In the ER membrane they assemble to large homo- and heterooligomeric complexes, which are located in special lipid domains called lipid rafts. The ERLIN complex was shown to facilitate the degradation of proteins via the endoplasmic reticulum associated degradation pathway, thereby negatively and positively regulating the stability of various proteins. In this way, the ERLIN complex regulates calcium channeling, cell cycle progression and the cholesterol homeostasis of the cell. Loss of function mutations in either *ERLIN1* or *ERLIN2* have been shown to cause the neurodegenerative disease hereditary spastic paraplegia in humans. The underlying pathogenic mechanism caused by mutations in *ERLIN1* and *ERLIN2* are not yet identified.

In the present work, I investigated the cellular functions of the ERLIN complex with two different cell models. In the first model, cells were lacking the ERLIN2 protein and were transiently downregulated for ERLIN1 to mimic an abrupt decrease in ERLIN complex function. In the second model, both ERLINs were removed by CRISPR/Cas9 gene editing, leading to the permanent deletion of the ERLIN complex. I found that the ERLIN complex is essential for maintaining a proper ER morphology. Loss of the ERLINs led to decreased ER-tubulation and a proliferation of ER-sheets to the cell periphery. The malformation of the ER is not induced by ER-stress or by differences in the lipid profile of the ER. It is rather attributed to the function of the ERLINs in regulating the stability of ER-shaping protein. Among others, the steady state protein levels of CLIMP63, an ER protein implicated in ER maintenance, are reduced due to decreased stability. This could be rescued by either treatment with the protease inhibitors 1.10. phenanthroline or Lopinavir, suggesting a regulatory role of the ERLINs in CLIMP63 cleavage. Furthermore, downregulation of the

metalloprotease ZMPSTE24 partially alleviated the decrease in CLIMP63 protein levels upon downregulation of ERLIN1. Tubulation of ERLIN DKO cells could be retrieved by treatment with Lopinavir, suggesting a protease dependent malformation of the ER. I propose that the ERLIN complex influences ER morphology, by regulating the abundance of proteins important for ER-shaping and distribution.